A FILTERABLE ORGANISM ISOLATED FROM THE TISSUES OF CHOLERA HOGS *

WITH PLATE 1

DANIEL J. HEALY AND EDWIN J. GOTT

From the Kentucky Agricultural Experiment Station, Lexington, Kentucky

Recently Healy and Smith[1] published a method whereby complement fixation may be obtained if a preparation of the mesenteric glands of the cholera hog be used as antigen. That this complement fixation is a specific reaction and not simply a reaction with the acetone insoluble residue, such as occurs in Wassermann's method, is strongly indicated in the work here reported.

Suitable mesenteric glands were selected from virus hogs, the glands carefully dissected out of mesentery, covered with 10 times their weight of absolute alcohol, and placed at 37 C. over night. The alcohol was then decanted off, the glands thoroughly ground with sterile sand, and the alcohol again added to the ground glands. The preparation was then placed at 37 C. for 8 days and thoroughly shaken each day. At the end of this period the preparation was filtered through an ordinary, white, folded filter paper. The filtrate, which measured 670 c.c., was evaporated by the aid of an electric fan to a somewhat pasty, deeply yellow mass. This mass was partly dissolved in 225 c.c. of ether and set aside to sediment. The ether was decanted from the sediment and allowed to evaporate to a volume of 15 c.c. To this 15 c.c. were added 75 c.c. (5 times its volume) of acetone. A heavy, flocculent precipitate immediately formed, which was allowed to settle, whereupon the supernatant liquid was decanted. Twenty-five cubic centimeters of methylic alcohol were added to the precipitate. The methylic alcohol dissolved a portion of the precipitate, leaving, however, a deeply yellow, sticky mass undissolved. After sedimentation the methylic alcohol, decanted and diluted 1 : 10 with normal salt solution (1.5 c.c.), was used as antigen in amounts varying from 0.01 c.c. to 0.03 c.c. with, in each case, 0.05 c.c. immune serum, 0.045 c.c. complement, 0.1 c.c. hemolysin, and 0.5 c.c. red blood corpuscles. The antigen, immune serum, and complement were mixed and placed at 37 C. for 1 hour, whereupon the hemolysin and corpuscles were added and the whole placed at 37 C. for 2 hours. Complete hemolysis followed in every case.

The test for complement absorption was negative. This test was repeated with 10 times the dose of antigen used in the first test, and the result was again negative.

In the light of these results it appears probable that our original antigen is specific. It is of interest that our original preparation of the mesenteric glands must remain for at least 8 days at a temperature

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[1] Jour. Infect. Dis., 1915, 17, p. 213.

of 4 C. for the antigen to develop, and it is of further interest that passage through an imported "F" Chamberland-Pasteur filter will remove the antigen from the preparation.

This preparation of mesenteric glands had been originally made in an effort to isolate a filterable organism from these glands. During the past two years we have made many such preparations and have endeavored to obtain a growth from them under a great variety of conditions—always, however, without success. On reviewing this work we noted that in the many attempts to obtain a growth, we had always filtered the preparation through an imported "F" Chamberland-Pasteur filter immediately after grinding the glands with sand. It therefore occurred to one of us (Healy) that a filterable organism might be present in such small numbers immediately after grinding and extraction of the glands that it could be readily filtered out by passage through an "F" bougie, but that if such a preparation was first placed at 4 C. for a period sufficient to allow some growth of the organism, and yet not long enough to exhaust its growth, and then filtered, it might be possible to pass an organism through the filter. This we succeeded in doing.

Mesenteric glands were obtained from 5 hogs which had been killed for the virus of acute cholera. The glands were carefully dissected from the mesentery and thoroughly ground with sand in an aseptic manner. To the ground glands was then added ten times their weight of a 1% glucose neutral beef broth, and the whole placed at 4 C. for 5 days. During this period an active fermentation occurred in this preparation. At the end of this period 100 c.c. of the supernatant liquid were passed through a reburnt, tested and sterile, imported "F" Chamberland-Pasteur bougie. The filtration required 40 minutes. The filtrate, which was brilliantly clear, was divided between 2 small, sterile Erlenmeyer flasks. One flask was placed in the Novy jar and the oxygen exhausted. The other flask was sealed with paraffin and placed in the air. Both flasks were placed at 4 C.

At the end of 4 days the flask placed in the air at 4 C. showed a distinct growth, which continued for about 2 weeks. This growth appeared as a fine sediment in the bottom of the flask. On the flask's being agitated with a circular motion, this sediment ascended through the fluid in the shape of a small cloud, resembling a delicate whiff of smoke. This is a characteristic appearance of cultures of this organism in fluid media. At the end of 13 days the flask placed in the Novy jar at 4 C. showed no evidence of growth. This flask was now sealed with paraffin and placed in the air at 4 C.; at the end of 7 days it

showed good growth, with the characteristic "whiff-of-smoke" appearance. This experiment was repeated 3 times, fresh virus glands being used for each experiment, and in each experiment we succeeded in obtaining the characteristic growth.

We demonstrated further that this filterable organism grows best at 37 C. in this extract of mesenteric glands, to which 5% sterile glycerin has been added. At this temperature the growth is comparatively rapid, and may be apparent at the end of 24 hours, reaching its maximal development in about 3 days. The organism also grows at 20 C. and 4 C., altho at these temperatures the growth is much slower and reaches its maximal development in about 2 weeks. The organism will not grow in the absence of oxygen, nor will it grow in any of the ordinary laboratory media, nor have we been able to obtain subcultures.

When the preparation of mesenteric glands is first made its reaction is very slightly acid. On standing at 4 C. this acidity increases in such manner that at the end of 5 days 100 c.c. of the preparation require about 4 c.c. of a N/N NaOH solution to neutralize it. It is of interest to note that the filterable organism will not grow in such a neutralized preparation.

A hanging drop preparation of this organism shows that it is nonmotile, and that it occurs in very small clumps, showing many minute, bright points. In such a preparation the individual organisms cannot be clearly distinguished. It appears that the individual organisms occur in small clumps and are surrounded by gelatinous material, so that great difficulties are encountered when one attempts to stain them. With the ordinary preparations of the aniline dyes the gelatinous material either stains deeply throughout or does not stain at all, and the individual organisms are not apparent. Under the proper conditions Giemsa's solution affords an entirely satisfactory stain.

A 3-day-old culture at 37 C. was centrifugated and the precipitate smeared on thoroughly cleaned microscopic slides and air-dried. The preparations were then fixed by inverting them in methylic alcohol for 1 hour, rinsed in distilled water, and air-dried. They were then stained by inverting them for 1 hour in a warm preparation of Giemsa's solution containing 50 drops of the stock solution in 50 c.c. of distilled water, exactly neutralized according to the method given by Mallory and Wright.[2] This solution was prepared immediately before using and was warmed on the water bath for 5 minutes. After staining, the preparations were washed in distilled water, air-dried, and mounted in xylol balsam.

[2] Pathological Technique, 1911, p. 428.

In such a preparation the organisms are clearly and distinctly visible as minute bodies, stained deep-lilac in color, the gelatinous material being pale-lilac in color. The organism appears as a coccus, or a very small bacillus, of about 0.2 to 0.3 micron in diameter. Figure 1 is a retouched photomicrograph of such a preparation.

In our original paper we demonstrated that the antigen is removed from the 1% glucose broth extract of mesenteric glands by passing the extract through an imported "F" Chamberland-Pasteur bougie.

In addition to cultivating this filterable organism and staining it in a satisfactory manner, we have succeeded in fixing complement with cultures of the organism.

A glycerin (5%) glucose (1%) beef broth culture which had grown at 37 C. for 11 days was centrifugated and the clear supernatant liquid used as antigen (Table 1). The test for complement absorption was negative.

TABLE 1

RESULTS OF COMPLEMENT-FIXATION TESTS USING THE CULTURE FLUID OF THE FILTERABLE ORGANISM AS ANTIGEN

Antigen in c.c.	Normal Salt Solution in c.c.	Immune Serum in c.c.	Complement in c.c.	Hemolysin in c.c.	Red Blood Corpuscles in c.c.	Hemolysis
.10	1.5	.05	.045	.10	.5	Complete
.13	1.5	.05	.045	.10	.5	Nearly complete
.15	1.5	.05	.045	.10	.5	Nearly complete
.17	1.5	.05	.045	.10	.5	Partial
.20	1.5	.05	.045	.10	.5	Very slight
.25	1.5	.05	.045	.10	.5	Complete
.30	1.5	.05	.045	.10	.5	Complete
	1.5	.20	.045	.10	.5	Complete

The organisms precipitated from this culture were suspended in 3 c.c. of normal salt solution and used as antigen in quantities of 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, and 0.08 c.c., but with negative results. In addition to this the supernatant liquid was again passed through an "F" Chamberland-Pasteur bougie and used as antigen, with negative results.

SUMMARY

From the work it is evident that we have obtained an organism from the mesenteric glands of hogs acutely ill with hog-cholera; that we have passed this organism through an imported "F" Chamberland-Pasteur bougie; that we have successfully cultivated and stained this organism; and that we have obtained complement fixation with the culture fluid in which this organism has grown, such fluid not being able to fix the complement previous to the growth of this organism in it. These studies are being continued.

EXPLANATION OF PLATE 1

Fig. 1. The filterable organism isolated from the tissues of cholera hogs. X 1080. Negative has been retouched.

Plate 1

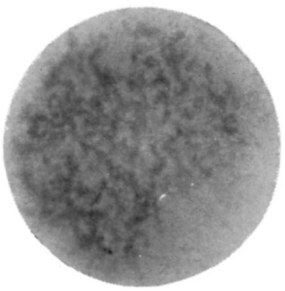

Figure 1